# Impact of stiffness over enteric neural progenitor cells

M.F. Sobstel[1] , R.D. Nanayakkara[2], Y. Zhang[1] , A.L. Bills[1] , P.C. Obidike[1] , M.A. Bednarczyk[3] , K.J. Lampe[2] , L.S. Cheng[1]
[1]University of Virginia, Department of Surgery, Charlottesville, VIRGINIA, USA; [2]University of Virginia, Department of Chemical Engineering, Charlottesville, VIRGINIA, USA; [3]University of Virginia, Department of Chemistry, Charlottesville, VIRGINIA, USA

UVA Children's

## Background

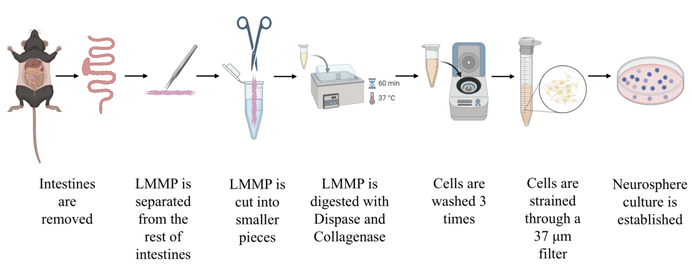
Hirschsprung disease is a congenital disorder where nerve cells are missing from the distal region of the colon. It is caused by the incomplete migration of enteric neural progenitor cells from cranial to caudal during development. As a result, those affected suffer from functional bowel obstruction, which requires surgical intervention. This usually consists of removing the aganglionic region of the colon and reattaching normal ganglionic intestine to the anus. Unfortunately, approximately 50% of Hirschsprung disease patients experience recurring problems after surgery. We believe that this might be caused by increased stiffness in the ganglionic part of the colon that remains with the patient after the surgery and was once considered normal. Previous studies in our lab found that stiffness in this part of the colon ranges between 20 and 25 kPa, while normal colons' stiffness is around 10 kPa and embryonic colon stiffness is around 1 kPa. It is unknown how this difference in stiffness affects enteric neurons.

**Aim:** Our goal was to investigate the relation between enteric neural progenitor cell viability and the stiffness of its environment.

## Materials and Methods

**LMMP isolation and neurosphere culture:** Male and female C57BL/6 mice aged 3–8 weeks were used in accordance with relevant ethical guidelines and regulations from our Institutional Animal Care and Use Committee protocols. The mice were housed in a controlled environment with a 12h light/dark cycle and provided *ad libitum* access to food and water. The longitudinal muscle with myenteric plexus (LMMP) was dissected from the small and large bowel, and enteric neural crest-derived progenitor cells (ENPC) were isolated in accordance with published protocols and propagated in culture as neurospheres. Experiments were performed with cells derived from primary neurospheres.

LMMP isolation and neurosphere culture




## Materials and Methods

Embedding the cells in NorHA-based hydrogels: After one week of culture, primary neurospheres have been dissociated and embeded in NorHA (Norbornene functionalized hyaluronic acid)-based hydrogels at a seeding dencity of $5 \times 10^6$ cells/ml. These hydrogels have been designed to have a specific stfifness, wchich was confirmed using rheological studies. The specific stiffness of the gels used for this experiment was 1, 10, 20 and 25 kPa to best reflect relevant biological environmental stiffness.

| **Stiffness** | 1 kPa | 10 kPa | 20 kPa | 25 kPa |
|---|---|---|---|---|
| **NorHA** | 1.5 wt% | 4 wt% | 6 wt% | 6 wt% |
| **Dithiothreitol crosslinking efficiency** | 30% | 30% | 25% | 40% |

**Viability testing:** After a week of culture in proliferation media, the gels have been stained for LIVE/DEAD cells (dyes used Calcein AM for live and Ethidium Homodimer-1 for dead) and imaged using Keyence BZ-X810 right after. Microscope images have been analyzed using ImageJ software, and statistical analysis was performed.

## Results

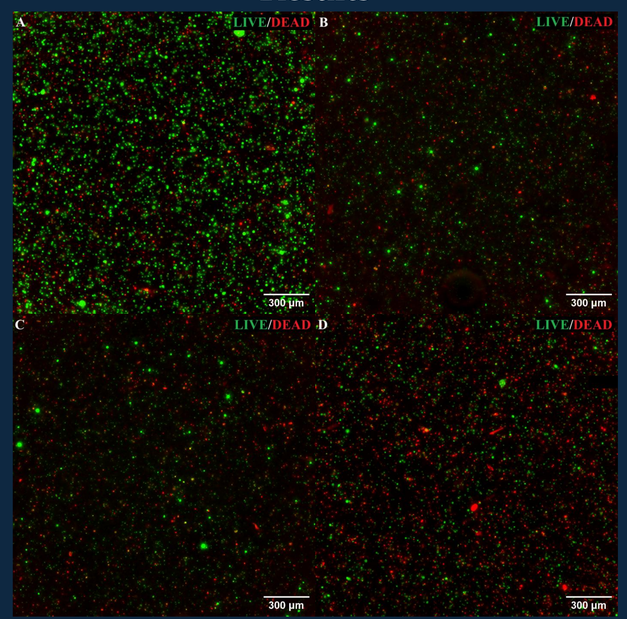



**Figure 1:** 4X magnification picture of LIVE/DEAD staining of cells embedded in hydrogels **A.** under simulated embryonic stiffness conditions (1 kPa). **B.** under healthy intestine simulated stiffness conditions (10 kPa). **C.** and **D.** under simulated Hirschsprung's disease stiffness conditions (respectively 20 kPa and 25 kPa).

## Results

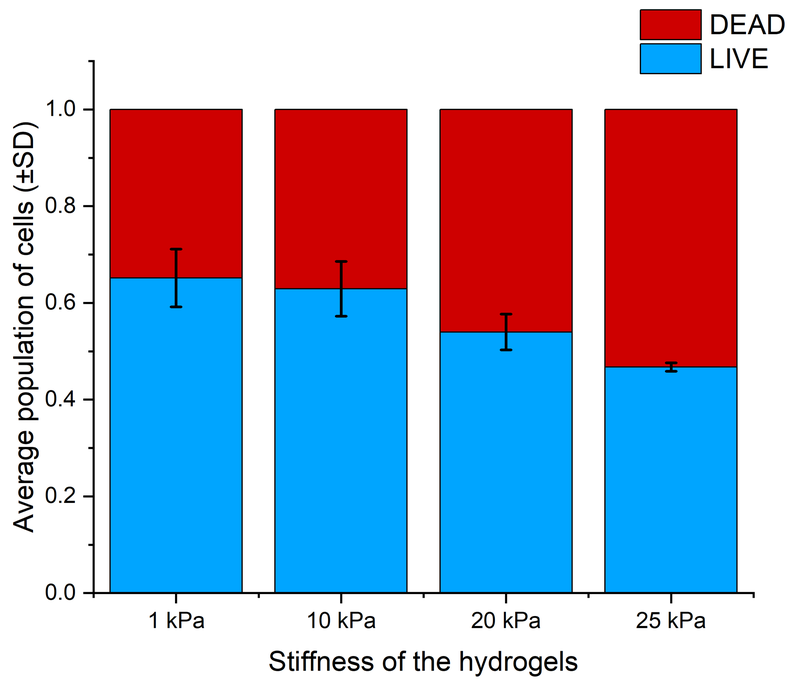



**Figure 2:** Viability and mortality of enteric neural progenitor cells under different stiffness conditions.

**Results:** Environmental stiffness had a statistically significant effect on the viability of enteric neural progenitor cells. Enteric neural progenitor cells cultured in 20 and 25 kPa gels had increased mortality and lowered viability when compared to cells cultured in 1 and 10 kPa gels.

## Conclusions and future plans

**Conclusions:** Our data suggests that increased stiffness has a negative influence on enteric neural progenitor cells. These findings suggest that gut stiffness may play a role in the pathology of Hirschsprung disease.

In future experiments, we hope to examine neural differentiation and migration in variable stiffness environments.

## Funding & Acknowledgements

Moneme C. et al. Activation of mechanoreceptor Piezo1 inhibits enteric neuronal growth and migration in vitro. DOI: 10.3389/fnmol.2024.1474025
**Funding:** Investigating the role of biomechanical forces on the enteric nervous system in Hirschsprung disease - NIDDK 5K08DK133673.